

## An antibody-lectin sandwich assay for the determination of CA125 antigen in ovarian cancer patients

R. MADIYALAKAN<sup>1</sup>, M. KUZMA<sup>1</sup>, A.A. NOUJAIM<sup>1</sup> and  
M.R. SURESH<sup>2\*</sup>

<sup>1</sup>*Biomira Research Inc. and* <sup>2</sup>*Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada, T6N 2N8*

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A two-step forward sandwich assay was developed for the determination of the ovarian tumour associated glycoconjugate antigen CA125 with anti-CA125 Monoclonal antibody B27.1 on the solid phase and <sup>125</sup>I-labelled wheat germ lectin as tracer in the solution phase. This Mab-lectin heterosandwich assay was optimized and the clinical utility was evaluated in sera from healthy volunteers and ovarian cancer patients. A correlation was established between Mab-lectin assay and the dual monoclonal antibody sandwich assay, TRUQUANT®OV2 RIA, that uses the same MAb B27.1 on the solid phase and a second <sup>125</sup>I-labelled B43.13 MAb in the solution phase. A potentially improved clinical utility is suggested for the Mab-lectin assay. The unique format seems to identify novel isoforms of CA125 with different carbohydrate side chains that would otherwise be undetectable in the MAb-MAb sandwich assay wherein the paratopes are likely directed to protein determinants.

**Keywords:** CA 125, antibody-lectin assay, ovarian cancer, wheat germ agglutinin

### Introduction

Cancer antigen CA125 is by far the most useful marker for detecting and monitoring patients with non-mucinous epithelial ovarian carcinoma [1, 2]. In a previous study, we described the generation of two distinct murine monoclonal antibodies, namely B27.1 and B43.13, reactive to different epitopes on the CA125 molecule [3–5]. In addition to these two antibodies developed in our laboratory and the original Mab OC 125 [1], several new Mabs (M2, M11, 130.22, 145.9, 602.1 and 602.6) that recognize different antigenic determinants of the same CA125 antigen molecule have also been reported [6–9]. Nearly two dozen anti-CA125 Mabs were reported at the first workshop on CA125 Mabs (TD-1, tissue differentiation-1) International Society of Oncodevelopmental Biology and Medicine in September 1994 under the auspices of the ISOBM, XXII, Groningen, Netherlands [9]. It has also been suggested that the combined use of antibodies reactive with different antigenic determinants will give

certain advantages to the detection of serum tumour markers [10, 11]. Thus, we have developed a two-step dual monoclonal antibody sandwich assay, TRUQUANT®OV2 RIA for the measurement of serum CA125 in cancer patients with MAb B27.1 on the solid phase and labelled MAb-B43.13 in the solution phase. In the recent CA125 workshop it was in fact observed [1] that the best sandwich assays were obtained by employing one MAb from Group A (OC 125-like) and one MAb from Group B (M11-like).

In the present study, we describe the development of a second generation CA125 assay that could potentially improve the clinical utility by identifying novel isoforms or microheterogeneity of CA125 with different carbohydrates side chains [12, 13]. This was accomplished by using an anti-CA125 Mab B27.1 on the solid phase to capture the CA125 molecule in the serum as the first step and after removing other serum glycoproteins by a wash step, detecting the carbohydrate moieties present in the antigen with a carbohydrate binding lectin. This format was also chosen instead of a solid phase lectin and solution phase MAb, because a MAb is more specific than a lectin probe.

\*To whom correspondence should be addressed.

## Materials and methods

TRUQUANT@OV<sub>2</sub> RIA kit was from Biomira Inc., Edmonton, Alberta, Canada. *Aleuria aurantia* lectin (ARA) was purified according to the published procedures [14]. Other lectins such as *Banderia simplicifolia* (BSII), *Canavalia ensiformis* (Con A), *Anguilla auguilla* (ANA), *Ulex europaeus* (UEA), *Limulus polyphemus* (LPA), *Phaseolus vulgaris* (PHA), *Triticum vulgaris* (WGA), *Arachis hypogea* (PNA) and *Lens culinaris* (LCA), were purchased from E.Y. Labs, San Mateo, USA and radiolabelled with iodogen and Na<sup>125</sup>I (Amersham, Canada) to specific activities in the range of 5–10  $\mu\text{Ci } \mu\text{g}^{-1}$  lectin. Sera from healthy controls and cancer patients with extensive clinical documentation were obtained from the serum bank of Biomira Inc.

CA125 used in this study was purified from ascites fluid obtained from an ovarian cancer patient by Sephacryl-S500 gel filtration chromatography. The void volume containing CA125 as measured in the TRUQUANT@OV<sub>2</sub> kit was pooled and the concentration of the antigen pool estimated by the same assay.

### Antibody-lectin sandwich assay

The assay was performed using polystyrene tubes coated with B27.1 MAb provided in the TRUQUANT@OV<sub>2</sub> RIA kit along with other reagents except that <sup>125</sup>I-B43.13 MAb was replaced with <sup>125</sup>I-labelled lectins. Briefly, the procedure included coating the polystyrene Sarstedt tubes with 1  $\mu\text{g}$  MAb B27.1 in 100  $\mu\text{g}$  PBS, blocking the tubes with 1% BSA/PBS and adding CA125 antigen provided as calibrators. A wash step removed the unbound serum proteins and glycoproteins. The bound CA125 was detected using 100  $\mu\text{l}$  <sup>125</sup>I-labelled lectin containing a mass of 10–20 ng. A standard curve was run with each assay using reference CA125 present in the kit. The CA125 levels are expressed as U ml<sup>-1</sup> serum. Since the same components of the commercial RIA kit were used, direct qualitative and quantitative comparisons could be made between the MAb-MAB vs Mab-lectin assays.

## Results

In our attempts to identify a lectin that could efficiently detect CA125 captured by solid phase MAb B27.1, several different labelled lectins were added to form the ternary complex and the results are shown in Table 1. Among the various lectins tested, wheat germ lectin alone could detect CA125 in a concentration dependent manner with comparatively low background values. No significant difference from background values were obtained with ARA, BSII, ConA, ANA, LPA and LCA. Some CA125 concentration dependent binding was observed with PNA, UEA and PHA lectins but their signal to noise ratios were not as good as the WGA. Hence, the latter was used for

**Table 1.** Binding of <sup>125</sup>I-labelled lectin to CA125 bound to solid phase B27.1 MAb.

Labelled lectin	DPM bound concentration of CA125 (U)			
	0	250	500	1000
ARA	3808	4052	3701	4334
BSII	346	371	380	755
Con A	16003	—	12835	17671
ANA	1322	898	1517	1035
UEA	1249	1365	2170	3171
LPA	709	687	725	710
PHA	6800	10020	10170	41935
LCA	23818	26427	25235	24981
PNA	399	946	1260	2387
WGA	2935	25394	34475	51857

Approximately 200,000 dpm of each labelled lectin was used with a specific activity in the range of 5–10  $\mu\text{Ci } \mu\text{g}^{-1}$  protein.

further evaluation in the development of the second generation lectin-MAB assay.

Figure 1 shows a comparative standard curve for the measurement of CA125 bound to MAb B27.1. The CA125 could not only be quantitatively detected in the Mab-lectin assay, the percentage bound at each concentration was also found to be higher compared to MAb-MAB sandwich assay. Experiments performed to standardize the assay conditions for CA125 measurement in the Mab-lectin assay suggested an incubation time of 3 h with 10 ng of lectin tracer to be optimal (data not shown). The MAb B27.1-wheat germ assay had good reproducibility with intra-and inter-assay coefficient of variation under 5%.

The clinical utility of the Mab-lectin assay was evaluated by measuring the CA125 levels in sera from 187 ovarian cancer patients. Control patients included 35 patients free of the disease and 50 healthy controls. The sensitivity of this assay for the detection of ovarian cancer was 88% (Fig. 2) when the upper limit of the normal range was taken as the value (100 U ml<sup>-1</sup>) that included 95% of the control sera.

The sensitivity of this assay is somewhat higher than that of MAb-MAB sandwich assay (80%) which has a normal cut off value of 37 U ml<sup>-1</sup> (Fig. 2). The specificity of the Mab-lectin assay was found to be 90% when sera from breast and prostate cancer patients as well as that of ovarian cancer patients free of the disease were studied for percent elevations in CA125 levels above the cut off value (Table 2). However, the specificity dropped to 70% when compared to lung cancer patients. This specificity was comparable to that of TRUQUANT@OV<sub>2</sub> RIA (data taken from the TRUQUANT@OV<sub>2</sub> RIA product literature). Generally, the absolute CA125 unit values for individual sera were

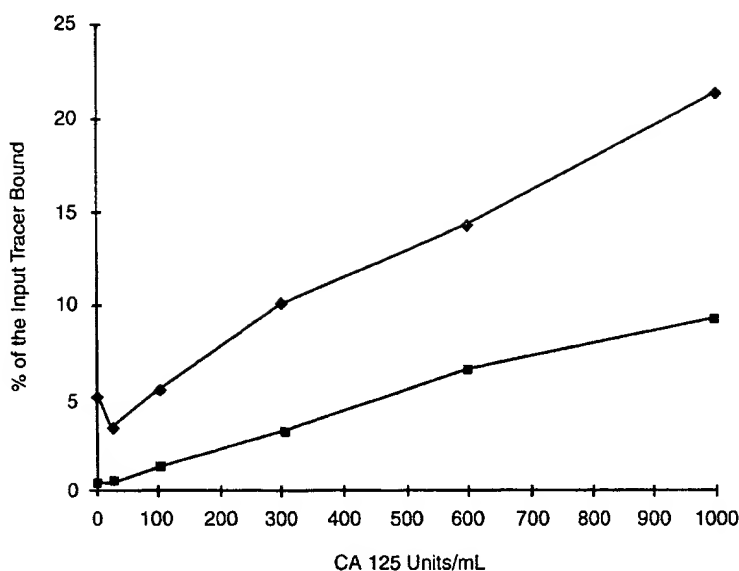


Figure 1. Standard curve for CA125 detected by wheat germ lectin or MAb-B43.13. ■ MAb-B43.13 ♦ Wheat germ Lectin.

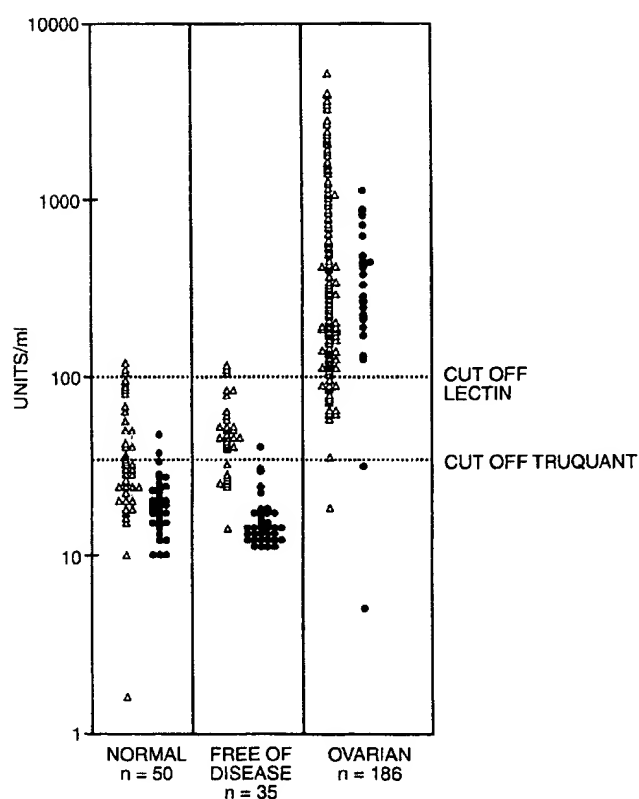


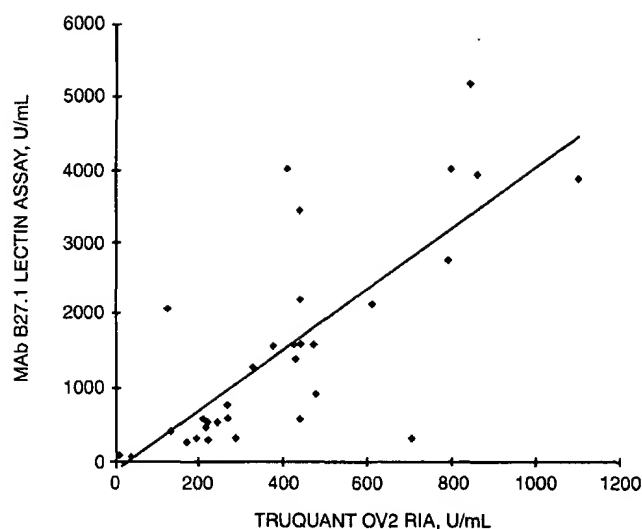
Figure 2. CA125 levels in ovarian patients with and without disease and healthy normals as measured by TruQuant@OV<sub>2</sub>™ RIA and MAb-B27.1/wheat germ lectin assay. Only 25 patients were measured in the TruQuant@OV<sub>2</sub>™ RIA and the *n* values reflect the total numbers measured for the MAb/lectin sandwich assay. Δ-MAb-lectin; ● TruQuant@OV<sub>2</sub> assay for CA125.

Table 2. Percentage positivity of various cancer types using CA125 values in the lectin-sandwich assay.

Cancer type	% positive	Number of patients studied
Breast	10	10
Lung	30	10
Prostate	10	10
Ovarian cancer patients free of disease	5	35

found to be higher in the MAb-lectin assay compared to the MAb-MAb assay, presumably a reflection of the repeated glycan determinants in the antigen and the general higher percentage of <sup>125</sup>I-WGA bound in the ternary complex compared to <sup>125</sup>I-B43.13 MAb used in the traditional dual monoclonal sandwich assay.

An overall correlation coefficient of 0.76 was observed between TRUQUANT@OV<sub>2</sub> RIA and B27-1/WGA assay (Fig. 3). This moderate correlation reflects glycan microheterogeneity which is prevalent in glycoproteins. However, good parallel clinical profiles were found between two assays when the progress of the disease was followed during treatment of individual patients by monitoring CA125 levels (Fig. 4). In fact, CA125 levels correlated well with clinical status of the patients, and the magnitude of the changes was sometimes more pronounced in the MAb-lectin assay. This could potentially provide an advantage over the dual monoclonal assay with increased sensitivity in monitoring the clinical course of ovarian cancer.

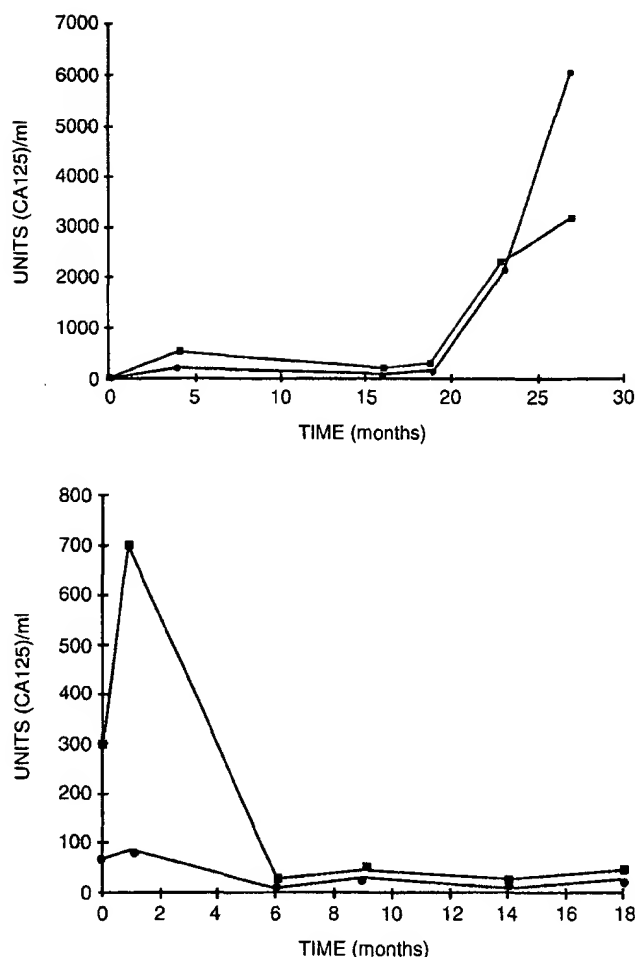


**Figure 3.** Correlation coefficient between MAb-Lectin assay and TruQuant®OV<sub>2</sub> assay for CA125. The correlation for the 32 patients was 0.76.

### Discussion

Oncogenesis is usually associated with alterations in cell surface carbohydrate structures, and tumour-associated glycoconjugate markers have been utilized in the diagnosis and treatment of human cancer using monoclonal antibodies and lectins [15–18]. CA125 is one of the key tumour-associated glycoconjugate antigens that has been established as a clinically useful marker for ovarian cancer [2]. This antigen is 24% carbohydrate by weight, making it possible to estimate this glycoprotein using lectins, and to detect isoforms or microheterogeneity in CA125 secreted by cancer cells as distinct from benign and normal CA125 antigen. Evidence for such a concept was observed in human chorionic gonadotropin (hCG) secreted by choriocarcinomas vs pregnancy [19]. In this instance hCG secreted by choriocarcinoma could be absorbed by *Datura stramonium* lectin unlike the isoform secreted in pregnancy.

Therefore, we set out to develop a new assay that could be specific for CA125 molecules associated with malignancy, by exploiting the carbohydrate composition of the antigen. This was accomplished through a sandwich assay using a MAb specific for CA125 namely MAb B27.1 on the solid phase and a carbohydrate binding lectin, wheat germ agglutinin as a tracer. This format (instead of a solid phase lectin) was chosen because serum has abundant glycoproteins and lectins are less specific than Mabs as the first step in a forward sandwich assay. From an applied perspective, it was also hoped that such a Mab-lectin sandwich assay would circumvent the dominant dual monoclonal sandwich assay patent held by Hybritech Inc.



**Figure 4.** CA125 level measured periodically in two ovarian cancer patients in TruQuant®OV<sub>2</sub> (●) and MAb lectin assays (■).

Lectins in combination with antibodies have been used for the detection of CA125, CA19.9 [20] CEA [21], alpha-feto protein [22] and Thomsen-Friedenreich antigen [23]. Among the different lectins tested, WGA exhibited a relatively low background and a good dose dependent signal to noise ratio for different amounts of CA125 captured by the MAb B27.1. The magnitude of the bound lectin probe in the ternary complex was higher than if a second Mab, such as MAb-B43.13, was used in the sandwich. This is not unexpected in view of the high carbohydrate content of CA125, and the likely existence of repeated carbohydrate determinants [24]. In addition the smaller size of the lectin and the broad spectrum glycan binding of WGA compared to antibody may also facilitate its binding to carbohydrate at multiple sites without steric hindrance.

The clinical utility of the Mab-lectin assay was evident since 88% of the patients with ovarian cancer had levels

above the normal values. The specificity of the MAb-lectin assay was found to be generally similar to that of the dual monoclonal assay, TRUQUANT®OV2 RIA. A reasonable correlation was also found between these two assays ( $r = 0.76$ ).

Improved clinical utility is suggested for the MAb-lectin assay as it could detect 88% ovarian cancer partials without compromising the specificity compared to 60–80% with MAb-MAb sandwich assay. Since the CA125 antigen has been shown to have more than 20 different oligosaccharides presented on the same molecule [25], use of a lectin could potentially improve the diagnostic power of the assay to detect ovarian cancer as suggested in this study.

The successful substitution of a lectin for a MAb also suggests that lectin-carbohydrate interaction is very similar to antigen-antibody interaction involving hydrophilic forces, Van der Waals contacts and hydrogen bonds as has been suggested recently [26].

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